

*Legionella*  
(615) 262-6362

## Introduction

*Legionella* are ubiquitous, fresh-water bacteria that infect humans sporadically or in epidemics. Although direct proof is lacking, transmission appears to occur primarily through airborne water droplets such as those from cooling towers or other heat-rejection systems. Laboratory data are relied on to confirm legionellosis because clinical signs are nonspecific. The organism can be isolated and identified from unfixed tissue and respiratory secretions or can be detected in fixed or unfixed tissue and body fluids with direct fluorescent antibody (DFA) staining. Culture is the recommended diagnostic procedure and should be attempted with other methodologies.

There are currently 34 *Legionella* species comprising 54 serogroups. Twelve of the species have been documented as etiologic agents of human disease by culture. Treatment decisions are based on identifying the genus since all species tested so far are susceptible to erythromycin and rifampin.

The Bacteriology Section offers culture and DFA staining of clinical specimens and reference cultures to public and private health care providers. Environmental samples that are directly related to a documented outbreak of legionellosis can be accepted for direct testing of *Legionella*.

Refer to INFECTIOUS DISEASES SEROLOGY, Section V for serological tests available.

**The Tennessee Medical Laboratory Act requires Laboratory Directors to submit *Legionella* species to the Tennessee Department of Health Laboratory for confirmation.**

## Specimen Collection

### Clinical Specimens

Recommended specimens for culture include respiratory tract secretions, tissues, sputum, pleural fluid, transtracheal aspirates, bronchial washings, and biopsies. **Do not use saline** to collect or dilute specimens for *Legionella* culture as the saline may inhibit growth. Use sterile broth or sterile distilled water.

Collect all specimens using aseptic technique following the protocols appropriate to the type of specimen. Place the specimen in a sterile screw-capped plastic centrifuge tube, such as the TB plastic conical tube. Seal the container securely to prevent leakage. Flexible plastic specimen collection cups are not acceptable for transporting specimens.

Prepare smears for DFA staining from respiratory specimens and formalized tissue by making thin films of respiratory fluids, sputum, or tissue homogenates on glass slides. Formalized tissue may be scraped gently with a sterile scalpel to obtain material for smear preparation. Alternatively, a cut edge may be pressed gently onto a glass slide. Smears should be air-dried, gently heat-fixed, or alcohol-fixed, flooded with 10% neutral formalin for 10 minutes, rinsed with distilled water, and air-dried. **Formalin fixation is required as a safety precaution.** Submit a minimum of four separate smears per specimen.

### Reference Specimens

Isolated organisms for identification of *Legionella* species should be pure cultures on charcoal-yeast extract agar slants incubated sufficiently to allow good visible growth. A single block of agar cut from fresh media may be placed in a sterile tube or on the surface of a charcoal agar slant.

### Environmental Specimens

Contact the laboratory about the proper method for submitting environmental samples.

### **Specimen Identification**

1. Complete all the provider and patient information areas on the Miscellaneous Exam Form PH-1573. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

### **Shipment of Specimens**

1. Ship tissue, sputum, and body fluid specimens **refrigerated** using Packing Instructions 650. Wrap the specimen in absorbent material. Place it in a leak-proof insulated container and pack with wet ice or freezer packs. If the specimen will not arrive at the laboratory within 3 days of collection, freeze the specimen and ship it on dry ice. Place the forms in plastic bags to prevent wetting or contamination.

Ship formalinized tissue, prepared slides, *Legionella* isolates, or environmental samples using Packing Instructions 602. Place the form in the outer container. Follow the shipping guidelines of your current carrier or method of shipment.

2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label to the outer container.
3. Ship to the Tennessee Department of Health Laboratory in **Nashville**.
4. Use first-class postage on US mail.
5. Contact the Bacteriology Section when an outbreak is suspected and **before** shipping the specimens.

## Legionella (Continued)

### Reporting and Interpretation of Results

Results of positive DFA examinations are telephoned immediately, followed by a mailed report, usually on the day of receipt or the following work day. Positive cultures are reported by telephone and by mail as soon as the growth is identified. Cultures are held for 10 days before being reported as negative.

*Legionella* is identified in culture and smears by specific DFA staining. DFA staining is a presumptive test. Cross-reactivity may occur among *Legionellaceae*.

Neither a negative DFA stain nor a negative culture rules out *Legionella* infection. Negative results may occur due to low numbers of organisms present, improper specimen or smear handling, and previous antimicrobial therapy.

*Legionella* isolates requiring definitive identification are forwarded to the Centers for Disease Control and Prevention.

Smears for <i>Legionella</i> from lung tissue are reported:	
Result	Report
25 or more strongly fluorescing * bacteria per smear	DFA presumptive positive
Less than 25 strongly fluorescing * bacteria per smear	The number of fluorescing bacteria per smear is reported.
No strongly fluorescing bacteria per smear	FA presumptive negative

Smears for <i>Legionella</i> from sites other than lung tissue are reported:	
Result	Report
5 or more strongly fluorescing * bacteria per smear	DFA presumptive positive
Less than 5 strongly fluorescing * bacteria per smear	The number of fluorescing bacteria per smear is reported.
No strongly fluorescing bacteria per smear	FA presumptive negative

\* Cells with morphology typical of *Legionella*.

## Legionella (Continued)

Culture results are reported as:
Negative for <i>Legionella</i> species.
Positive for <i>Legionella</i> species.

The results of all specimens are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the TDH Communicable and Environmental Disease Services and the health department in the county where the patient lives.

### Criteria for Unacceptable Specimens

#### All specimens

1. The specimen not properly identified with the patient's name and the tear strip control number.
2. The patient identifier on the specimen did not exactly match the identifier on the form.
3. The specimen or the slide was broken in transit.

#### Clinical specimens

1. The specimen was shipped unrefrigerated or unfrozen.

#### Environmental samples

1. The environmental sample was not directly related to a documented outbreak of legionellosis.

#### Reference specimens

1. The specimen was non-viable.
2. A mixed specimen was submitted.

## Legionella (Continued)


### Miscellaneous Exam Form PH-1573

#### FRONT

SOCIAL SECURITY NO.		TENNCARE NO.		MCO		MISCELLANEOUS EXAM		A 349473	
MEDICARE NO.				RECORD FOLDER NO.				DATE REPORTED	
PATIENT'S NAME - LAST, FIRST, MIDDLE				SPOUSE - FIRST NAME				DATE/TIME RECEIVED	
STREET AND NUMBER						COLLECTION DATE		DATE OF ONSET	
TOWN				STATE		ZIP		LAB NO. ▼	
DATE OF BIRTH		RACE		ETHNICITY		SEX		PHONE NO.	
COUNTY NO.		COUNTY NAME				SITE NO.		SOURCE: <input type="checkbox"/> BLOOD <input type="checkbox"/> URINE <input type="checkbox"/> FECES <input type="checkbox"/> THROAT <input type="checkbox"/> OTHER	
PATHOGEN/DISEASE SUSPECTED/SYMPTOMS									
EXAMINATION REQUESTED									
RECENT TRAVEL 1 <input type="checkbox"/> YES 2 <input type="checkbox"/> NO STATE/COUNTRY									
EXAMINATION RESULTS									
<input type="checkbox"/> SUBMITTED TO REFERENCE LABORATORY FOR EXAMINATION. RESULTS FORTHCOMING.									
EXAMINED BY: RDA-1160									

PLEASE FILL OUT SHADED AREA COMPLETELY  
 TN. DEPT. OF HEALTH DIV. OF LAB SERVICES  
 PRINTED BY STACHARD REGISTER U.S.A. 2004

REPORT  
 TO  
 NAME  
 ADDRESS  
 CITY  
 STATE  
 ZIP CODE


 TENNESSEE DEPT. OF HEALTH  
 LABORATORY SERVICES  
 MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR

☐ K ☐ J ☐ N  
 LABORATORY PERFORMING EXAMINATION

#### BACK

#### TIGHTEN CAPS SECURELY SUBMIT IN DOUBLE MAILING CONTAINER

#### STOOL CULTURES FOR SALMONELLA, SHIGELLA, CAMPYLOBACTER, AND E. COLI 0157

Place two (2) cotton tipped swabs dipped into feces or other specimens and insert into Amies, Stuarts, or Cary-Blair transport medium. Submit the transport medium refrigerated within two (2) days of collection.

**INTESTINAL PARASITES:** Place amount of feces equal to volume of formalin in container designed for intestinal parasites (5% Formalin)

**CULTURE FOR IDENTIFICATION** - Submit pure cultures on non-selective media such as trypticase soy agar slants or enriched slants (Blood or Chocolate) when required.

**ANAEROBIC ORGANISMS** - Submit in semi-solid media such as thioglycollate, overlaid with sterile vaspar to prevent exposing culture to oxygen.

#### TESTING LABORATORY LOCATION CODES

J = JACKSON BRANCH LAB, 295 SUMMAR DRIVE, P.O.BOX 849, JACKSON, TN 38302-0849 - DR ORISTYNE WALKER, DIRECTOR  
 K = KNOXVILLE BRANCH LAB, 1522 CHEROKEE TRAIL, P.O.BOX 59019, 37950-9019, KNOXVILLE, TN- DR MICHAEL W. KIMBERLY, DIRECTOR  
 N = NASHVILLE REFERENCE LAB, 630 HART LANE, NASHVILLE, TN 37247-0801 - DR MICHAEL W. KIMBERLY, DIRECTOR

***Streptococcus* Group A**  
(615) 262-6362

**Introduction**

The principal streptococcal species producing communicable disease in humans, Lancefield Group A streptococci or *Streptococcus pyogenes*, is primarily a resident of the pharynx. This microorganism causes a variety of respiratory tract diseases, including pharyngitis, rhinitis, tonsillitis, pneumonia, and scarlet fever. Group A streptococci are found in pyoderma and impetigo lesions, in wound infections, and in the blood of patients with erysipelas, cellulitis, and septicemia. Nonsuppurative disease, such as acute rheumatic fever and acute glomerulonephritis, may follow streptococcal pharyngitis.

Specimens for the isolation of *Streptococcus* Group A are accepted from public health providers. The Nashville laboratory performs a latex agglutination to identify the organism, while the direct fluorescent antibody (DFA) procedure is used to identify *Streptococcus* Group A by the regional laboratories.

Reference cultures are accepted from public and private health care providers for the confirmation of *Streptococcus* Group A. In cases of systemic infections or necrotizing fasciitis or if acute rheumatic fever or glomerulonephritis are suspected, referred cultures will be forwarded to the Centers for Disease Control and Prevention for "M" and "T" typing.

**The Tennessee Department of Health requests that laboratory directors submit isolates of *Streptococcus* Group A from a sterile site to the TDH Laboratory for surveillance purposes.**

**Specimen Collection**

**Throat cultures**

Rub the tonsils and pharynx with a swab while avoiding the tongue or uvula tissues. Touch any visible exudate with the swab. (The two most common pitfalls that result in inadequate specimens are swabbing the tongue or uvula tissues rather than the pharynx and inadequate exposure of the pharynx.)

**Nasal culture**

Take nasal cultures with sterile cotton-tipped flexible wire nasopharyngeal swabs specifically designed for this purpose. Raise the tip of the nose with one hand and introduce the swab along the floor of the nasal cavity under the middle turbinate until you reach the pharyngeal wall. Do not use force. If any obstruction is encountered, do not take the nasopharyngeal culture on that side.

**Skin culture**

Collect skin lesion specimens by removing the crust of the pustule or vesicle. Rub a sterile swab, firmly but gently, into the lesion. This may cause some discomfort to the patient, but it is important to ensure maximal recovery of streptococci.

**Wound cultures**

Collect wound cultures in the same manner as skin cultures.

**Inoculate the specimen directly on the surface of trypticase soy agar (TSA) slants.** Discard the swab. Ship it to the laboratory immediately. If a delay in shipment is anticipated, incubate the culture at 35 to 37°C for no longer than 24 hours or hold at room temperature.

## Streptococcus Group A (Continued)

### Specimen Identification

1. Complete all the provider and patient information areas of the Group A *Streptococcus* Form PH-1587. Include pertinent clinical information with each specimen.
2. Using indelible ink, label each specimen with the date of collection and the patient's first and last name. Attach the control number on the tear strip to the specimen, and secure it with transparent tape. Unlabeled specimens or specimens where the patient identifier on the specimen does not exactly match the identifier on the form will not be tested.

### Specimen Shipment

1. Place the form in the outer container. Ship at ambient temperatures. Follow the shipping guidelines of your current carrier or method of shipment.
2. Affix the mailing label (PH-0838), return address, and infectious substance (etiologic agent) or clinical (diagnostic) specimen label on the container.
3. Ship to the TDH Laboratory in **Jackson, Knoxville or Nashville**.
4. Use first-class postage on US mail.

### Reporting and Interpretation of Results

Specimens received before 8:30 AM will be tested the day received. Specimens that do not have visible growth when received or that have to be repeated will be reported within 2 to 3 working days.

Performed by the Nashville Laboratory Services, a positive result with latex agglutination is determined by characteristic clumping of the latex reagent with the specimen. Viable and nonviable organisms will be detected.

Specimens are reported
Positive: Group A Streptococci found by latex agglutination.
Negative: Group A Streptococci not found by latex agglutination.

Presently performed by the regional laboratories, a positive result with the direct fluorescent antibody test is determined by the presence of three or more chains of organisms with typical cellular morphology and intense fluorescence. Viable and nonviable organisms will be detected.

Specimens are reported
Positive: Group A Streptococci found by fluorescent antibody.
Negative: Group A Streptococci not found by fluorescent antibody.

Results are reported to the health care provider who submitted the specimen. In addition, positive results are reported to the health department in the county where the patient lives.

## Streptococcus Group A (Continued)

### Criteria for Unacceptable Specimens

1. The specimen was not properly identified with the patient's name and/or the tear strip control number.
2. The patient identifier on the specimen does not exactly match the identifier on the form.
3. The specimen was broken in transit.
4. The media had expired or was dehydrated.
5. There was no growth on the specimen. (The specimen is incubated in the laboratory for 24 hours before reporting. No growth indicates that the slant was not inoculated.)

### Group A Streptococcus Form PH-1587 FRONT

DO NOT DETACH GROUP A STREPTOCOCCUS		PRINTED BY STANDARD REGISTER U.S.A. ZPSE 8		PLEASE FILL OUT SHADED AREA COMPLETELY		A0370275	
SOCIAL SECURITY NO.		TENN CARE NO.		MCO		GROUP A STREPTOCOCCUS	
MEDICARE NO.		RECORD FOLDER NO.		DATE REPORTED		DATE/TIME RECEIVED	
PATIENT'S NAME - LAST, FIRST, MIDDLE		SPOUSE - FIRST NAME		COLLECTION DATE		DATE OF ONSET	
STREET AND NUMBER		TOWN		STATE		ZIP	
DATE OF BIRTH		RACE		ETHNICITY		SEX	
COUNTY NO.		COUNTY NAME		PHONE NO.		SITE NO.	
NAME		ADDRESS		CITY		STATE	
ZIP CODE		K		J		N	
PH-1587 REV 4/00		TENNESSEE DEPT. OF HEALTH LABORATORY SERVICES MICHAEL W. KIMBERLY, DR. P.H., DIRECTOR		LABORATORY PERFORMING EXAMINATION		EXAMINED BY:	
ANTIBIOTIC THERAPY		1 <input type="checkbox"/> YES		2 <input type="checkbox"/> NO		WHEN	
SOURCE OF SPECIMEN		1 <input type="checkbox"/> THROAT		2 <input type="checkbox"/> NASO-PHARYNGEAL		3 <input type="checkbox"/> OTHER (specify)	
EXAMINATION RESULTS		1 <input type="checkbox"/> GROUP A STREPTOCOCCI FOUND BY FLUORESCENT ANTIBODY.		2 <input type="checkbox"/> GROUP A STREPTOCOCCI NOT FOUND BY FLUORESCENT ANTIBODY.		3 <input type="checkbox"/> UNSATISFACTORY	
RDA-1160							

BACK

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## Water Microbiology (615) 262-6362

### Introduction

The Environmental Microbiology Section performs microbiological analyses of water and wastewater samples. As the only direct measures of pollution by man and other warm-blooded animals, microbiological parameters contribute unique information on water and wastewater quality and the public health risk from waterborne disease. Samples are tested for the presence or absence of the coliform group of bacteria, which are indicators of fecal contamination. Microbiological analyses are conducted to:

- Determine the safety of public drinking water supplies.
- Monitor ambient water quality for recreational, industrial, agricultural, and water supply uses.
- Monitor municipal and industrial discharges.
- Identify the sources of bacterial pollutants.
- Evaluate water resources.

The Tennessee Department of Health (TDH) Laboratory accepts samples from both public and private water systems. The procedures used are those specified in *Standard Methods for the Examination of Water and Wastewater* and EPA's *Microbiological Methods for Monitoring the Environment*. The laboratory uses the chromogenic substrate coliform test procedure for total coliform, and *Escherichia coli*; and test to detect *Enterococcus*. The laboratory uses the membrane filtration procedure for fecal coliform and fecal streptococcus in wastewater. These methods provide the most probable number of bacterial colonies in a 100mL sample.

The most probable number (MPN) procedure estimates the number of specific organisms in water and wastewater by the use of probability tables. The MPN method is an alternative to the Colilert procedure and is used for total and fecal coliform tests primarily with problem water that might contain sediments, sludges, or muds.

The tests commonly performed include:

Community water supplies	Total coliform and <i>Escherichia coli</i>
Non-community water systems	Total coliform and <i>Escherichia coli</i>
Private waters	Total coliform and <i>Escherichia coli</i>
Swimming pools	Total coliform and <i>Escherichia coli</i>
Natural swimming areas	Fecal coliform <i>Escherichia coli</i> and <i>Enterococcus</i>
Wastewater effluents	Fecal coliform <i>Escherichia coli</i> , <i>Enterococcus</i> , and (fecal streptococcus upon request)

Water samples are tested for pathogens when documentation for isolation of the specific organism is present and water had been epidemiologically indicated. For additional information on this testing, contact the Environmental Microbiology Section.

### Distribution of Water Sample Collection Bottles

**Community Water Supplies:** The TDH Laboratory ships water sample bottles to each water supply facility monitored under the Safe Drinking Water Act. The Tennessee Department of Environment and Conservation (TDEC) Division of Water Supply determine the number of bottles each water facility needs.

**Non-Community Water Systems:** The TDH Laboratory ships sample bottles to each system being monitored. One bottle is sent monthly or quarterly as determined by the TDEC Division of Water Supply.

## Water Microbiology (Continued)

### **Private drinking water supplies, swimming pools, natural swimming areas, etc.:**

Bottles are provided to local health departments and the Tennessee Department Agriculture for support of programs that are not covered by the Safe Drinking Water Act.

### **Sample Collection (Standard method 9060)**

1. A qualified environmentalist or a certified employee of a water treatment plant must collect the samples by the procedures designated in the current edition of *Standard Methods for the Examination of Water and Wastewater*.
2. Collect the sample in a water sample bottle (a sterile 4-oz polypropylene screw-cap bottle containing a dechlorinating and chelating agent). Do not lay the cap down. Be extremely careful not to allow anything to touch the inside of the sample bottle or the bottle cap.
3. Collect 100 ml by filling to the fill line at the shoulder of the bottle. Recap and mix by shaking the sample.

### **Sample Identification**

1. Complete the Water Sample Request Form PH-1575. The form must contain:
  - Water system name, address, telephone number, county, and location where the sample was collected.
  - Date and military time (see Appendix 2) of collection.
  - Chlorine residual.
  - Sample collector's name and title.
  - Sample type/source.
  - Examination requested.
  - Public water system identification number (PWS ID), if applicable.

The form also has an ID number that can be removed and attached to the bottle for additional sample identification.

The procedure for collection of the water sample, filling out form, and the sample type key is on the back of the Water Sample Request Form. The key for the sample type is:

D-Distribution  
R-Repeat  
N-New Lines  
S-Special  
Q-Quality Control

### **Shipment of Sample**

1. Place the form around the water sample bottle and place the sample in a mailing container. Follow the packing Instructions 650, Section I-18.
2. Affix the mailing label (PH-0838) and the return address to the container.
3. Ship the sample to the TDH Laboratory in **Jackson, Knoxville, or Nashville**.
4. Use first-class postage on US mail.
5. The holding/transit time between sampling and examination for total coliform analysis must not exceed 30 hours. If possible, samples should be refrigerated (4 to 10°C) during transit. Mail samples so that they do not arrive at the laboratory on holidays or weekends.

## Water Microbiology (Continued)

6. Samples for *Escherichia coli*, Fecal coliform, or *Enterococcus* analysis must be refrigerated from the time they are collected until they are analyzed and must arrive in the laboratory within 6 hours after collection.

### Reporting Procedures and Interpretation of Results

A water analysis report refers only to the sample as received. It should not be regarded as a complete report on the water supply.

The turn around times for tests performed by the TDH Laboratory are:

Test	Negative Sample	Positive Sample
Total Coliform, Colilert	1 day	1 day
Total Coliform, MPN	3 days	7 days
Fecal Coliform, MF and MPN	3 days	5 days
<i>Enterococcus</i> , Enterolert	1 day	1 day
<i>Escherichia</i> , Colilert	1 day	1 day
Fecal Streptococcus	2 days	2 days

### Results are reported

	Total coliform	<i>Escherichia coli</i>	<i>Enterococcus</i>	Fecal coliform	Fecal Streptococcus
Negative Results	Negative for total coliform	Negative for <i>Escherichia coli</i>	Negative for <i>Enterococcus</i>	Negative for Fecal coliform	Negative for fecal streptococcus
Positive Results	Positive for total coliform	Positive for <i>Escherichia coli</i>	Positive for <i>Enterococcus</i>	Positive for Fecal coliform	Positive for fecal streptococcus
Method Used	SM 9223 SM 9221B	SM 9223 SM 9221B	Enterolert	SM 9221E SM 9222D	SM 9230 (with prior notification)

**If coliform bacteria are present in a water sample, the water is considered unsafe for drinking purposes.** The water supply must meet the following requirements for positive samples:

**Community and non-community water systems:** Phone calls are made on all positive water samples. The supply facility must submit 4 repeat samples immediately.

**If the result of the sample is unsatisfactory, a new sample must be submitted immediately.**

Results of all tests are reported to the provider. Results from community supplies and non-community water systems are reported to the TDEC Division of Water Supply and the provider's Water Pollution Basin Office. Depending on the source of the sample, results from other providers are reported to any required official agency. (Examples of such agencies include the

**Water Microbiology (Continued)**

### Criteria for Unacceptable Specimens

- # Water Sample Request Form PH-1575
- ## FRONT

**BACK**